1,099,865

PATENT SPECIFICATION

NO DRAWINGS

1,099,865

Date of Application and filing Complete Specification: June 1, 1966.

No. 24375/66.

Application made in United States of America (No. 494,894) on Oct. 11, 1965.

Complete Specification Published: Jan. 17, 1968.

Index at acceptance:—C2 C(1Q4, 1Q6C, 1Q7A, 1Q8A, 1Q8B, 1Q8C, 1Q8D, 1Q9B, 1Q9L, 1Q11G); A5 E(1A4B2, 1A4B3, 1A4B4, 2); A5 R(83H, 83K); C5 D(6B4, 6B8, 6B10C, 6B12A, 6B12B1, 6B12B2, 6B12E, 6B12K2, 6B12M); D2 B13X

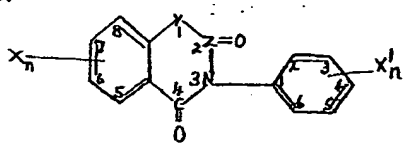
Int. Cl.:—C 07 d 87/02, C 07 d 93/08, C 07 d 95/00

COMPLETE SPECIFICATION

Benzoaziniediones and Germicidal Compositions made therewith

We, STECKER INTERNATIONAL S.P.A., a body corporate organised under the laws of Italy, of Via Turati No. 29, Milan, Italy, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to the preparation of new benzoaziniediones, including benzothioxaziniediones and benzoxaziniediones, and to novel germicidal compositions prepared therewith. The compounds which are the subject of the present invention fall within the generic formula:



where X and X¹ are chlorine, bromine, iodine or CF_3,

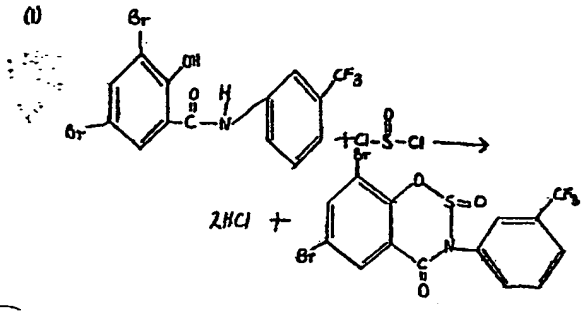
n is an integer from 0 to 3, subject to the proviso that X or X¹ represent at least one and not more than two CF_3 groups,

Y is sulphur or oxygen, and

Z is sulphur or carbon.

The small numerals within the nuclei are inserted merely for more convenient orientation of the derivatives to be discussed herein.

The compounds of the present invention may be prepared by reacting a substituted salicylanilide with thionyl chloride, phosgene, or ethyl chloroformate according to the following typical reactions:



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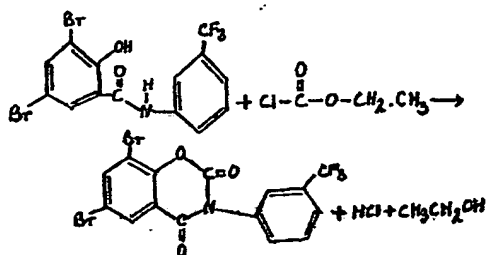


In reaction (1) 3,5-dibromo-3'-(trifluoromethyl) salicylanilide is reacted with thionyl chloride to produce 6,8-dibromo-3-(3-trifluoromethylphenyl)-1,3-benzothioxazine-2,4-dione. In reaction (2) the same salicylanilide is reacted with ethyl chloroformate to produce 6,8-dibromo-3-(3-trifluoromethylphenyl)-1,3-benzoxizine-2,4-dione.

These compounds may be prepared according to the method described by Stanseth, Baker and Roman, J. Med. Chem., *6*, 1212 (1963). A typical method of preparation is as follows:

6,8-Dibromo-3-(3-trifluoromethylphenyl)-1,3-benzoxazine-2,4-dione.

A molal solution of 3,5-dibromo-3'-(trifluoromethyl) salicylanilide in a mixture of pyridine and acetonitrile is stirred at 2—5°C. during dropwise addition of a molal quantity of ethyl chloroformate. Stirring is continued for 1—2 hours while the temperature is gradually increased to 120°—125°C. After about 60 mls. of distillate has been collected in a Barrett trap, the mixture is slowly cooled and, before it is solidified, water and concentrated HCl are added with stirring and further cooling. The crude product is then isolated, washed with water, and air-dried. The compound may be recrystallized from acetone, after decolorization with activated charcoal. The recrystallized product is then recovered.

Table I gives a list of compounds which have been prepared in accordance with the foregoing method.

TABLE I

No.	Salicylanilide	Reactant	Product	Properties
1.	3,5-Dibromo-3′-(trifluoromethyl)	$SOCl_2$	6,8-dibromo-3-(3-trifluoromethyl-phenyl)-1,3-benzothioxazine-2,4-dione	m.p. 190—5 °C.
2.	3,5-Dibromo-3′-(trifluoromethyl)	Ethyl chloroformate	6,8-dibromo-3-(3-trifluoromethyl-phenyl)-1,3-benzoxazine-2,4-dione	m.p. 233—5 °C.
3.	3′-(trifluoromethyl)	Ethyl chloroformate	3-(3-trifluoromethylphenyl)-1,3-benzoxazine-2,4-dione	m.p. 198—199 °C.
4.	2′-Chloro-3′-(trifluoromethyl)	Ethyl chloroformate	3-(2-chloro-3-trifluoromethylphenyl)-1,3-benzoxazine-2,4-dione	m.p. 195—198 °C.
5.	3,5-Diodo-3′,5′-bis(trifluoromethyl)	Ethyl chloroformate	6,8-diiodo-3-(3,5-bis(trifluoromethyl-phenyl)-1,3-benzoxazine-2,4-dione	m.p. 214—8 °C.
6.	2-Thiophenyl-3,5-dibromo-3′-(trifluoromethyl)	Ethyl chloroformate	6,8-dibromo-3-(3-trifluoromethyl-phenyl)-1,3-benzothiazine-2,4-dione	m.p. 238—40 °C.
7.	3,5-Dichloro-4-(trifluoromethyl)-4′-iodo	Ethyl chloroformate	6,8-dichloro-7-(trifluoromethyl)-3 (4-iodophenyl)-1,3-benzoxazine-2,4-dione	m.p. 220—4 °C.

The compounds of the present invention have been found to show unexpectedly high toxicity to micro-organisms, such as bacteria, fungi, and similar growths, as compared to the unsubstituted or heretofore known compounds. The antibacterial activity of the present compounds is shown in Table II, as the minimum inhibitory concentration (MIC), against *Staphylococcus aureus*. A 24-hour broth culture of each organism was made in Brain Heart Infusion (BHI) broth.

A number of screw cap test tubes, each containing 9 mls. of BHI broth, were prepared and sterilized for 15 minutes at 25 psi at 120°C. A number of 100 ml. volumetric flasks, each containing about 80 ml. of BHI broth also were prepared, were capped with glass caps, and were sterilized in the same manner as were the test tubes.

One tenth of a gram of the compound to be tested was accurately weighed and was dissolved in acetone or alcohol. The mixture then was transferred with aseptic technique to the previously sterilized volumetric flasks containing the BHI broth. With aseptic technique, the mixture was brought up to 100 ml. with BHI broth. This mixture then consisted of 1:1000 dilution of the compound to be tested.

Ten ml. of this mixture were transfered aseptically to a sterile capped tube by a 10 ml. Mohr pipette. Serial dilutions then were made from this stock solution with concentrations of 1:10,000, 1:100,000, 1:1,000,000 and 1:1,100,000,000 of the compounds.

To each of the dilutions of a given compound then were added 0.1 ml. of a 24-hour broth culture of the organism to be tested. The turbidity of the broth solutions was determined by a Welsh Densichron. The densitometer was chosen over visible observation for purposes of accuracy when end points were questionable. The broth dilutions were then allowed to stand for 24 hr. at 37°C. A control consisting of 0.1 ml. of a 24-hour broth culture and 9 ml. of BHI broth also was prepared and was subjected to the same conditions as the compounds to be tested. At the end of the 24-hour period, the tubes again were observed with the densitometer. If growth occurred, it would be manifested by an increase in turbidity in the broth.

All compounds were subected to the same testing and their antibacterial activities were compared.

The unsubstituted compound No. 1 of Table 2 was included to show its relative ineffectiveness, as compared to the compounds of the present invention. The expression "germicidal or antibacterial activity" includes inhibiting and killing action against bactera, fungi and similar organisms. The compounds of the present invention have been found effective against organisms such as *S. typhi*, *B. coli*, *L. casei*, and others.

The present germicides are useful in compositions comprising one or more compounds of the present invention and a germicidally inert material, i.e., relatively speaking, such as an inert pharmaceutical diluent, soap and/or detergent, and plastics and/or rubber. Fibrous materials may also advantageously be impregnated with one or more compounds of the present invention. For example, some soaps and detergents possess a bactericidal action, but such action, relative to those of the compounds of the present invention, is weak and of little effect in comparison with the overall germicidal activity of the composition. In such compositions, the compounds of the present invention may be employed in concentrations as low as 10 p.p.m., although, from a practical point of view, it is desirable to use as much as 50 p.p.m. or 0.001% by weight, or 0.01%, 0.1%, 0.5% or as much as 1% or 5%, or even more.

Particularly useful compositions of the present invention are those comprising soaps and detergents, and especially toilet soaps of cosmetic detergents in which the compounds of the present invention may be employed in concentrations of 0.01%, 0.1%, 0.5% or even up to 1% by weight, or more. The term "detergent" employed herein will be used to include all synthetic and natural cleansing compositions, including cationic detergents, such as dimethyl stearamido-propyl-2-hydroxy-ammonium dihydrogen phosphate, anionic detergents, such as commercial soaps, e.g., alkali metal soaps of hydrolyzed natural or synthetic glycerides of fatty and similar acids, e.g., sodium and potassium stearates or oleates, ampholytic detergents, such as sarcosine, non-ionic detergents, such as polyoxypropylene polyoxyethylene condensates, natural detergents, such as starches, vegetable gums, and the mixtures thereof. The term "soap" employed herein is used in its popular or ordinary meaning, i.e., a cleansing composition prepared from an alkali metal compound, such as potassium or sodium hydroxide and a fat or fatty acid, both saturated and unsaturated.

Another valuable use of the compounds of the present invention is the use thereof to sanitize fibrous material, such as cotton gauze, dressings, textiles, and paper pulp, preferably in concentrations of about 0.01% to 0.5% by weight. They also serve as antiseptic agents, when incorporated in plastic or rubber compositions, prior to

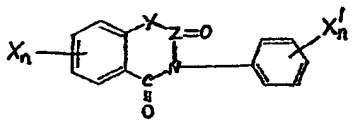
molding into articles of commerce, such as baby rattles, gloves, and food wrappers, preferably in concentrations of 0.005% to 0.5% by weight.

TABLE II

No.	Compound	Effectiveness Against S. aureus MIC × 10^3
1.	3-Phenylbenzoxazine-2,4-dione	1:1 — 1:10
2.	6,8-Dibromo-3(3-trifinoromethylphenyl)-1,3-benzoxazine-2,4-dione	1:1000 — 1:10,000
3.	3-(3-Trifluoromethylphenyl)-1,3-benzoxazine-2,4-dione	1:100 — 1:1000
4.	3-(3-Trifluoromethyl-2-chloro-phenyl)-1,3-benzoxazine-2,4-dione	1:1000 — 1:10,000

WHAT WE CLAIM IS:—

1. Compounds having the general formula:



where:

X and X^1 are chlorine, bromine, iodine, or CF_3,

n is an integer from 0 to 3, subject to the proviso that X or X^1 represent at least one and not more than two CF_3 groups,

Y is sulphur or oxygen, and

Z is sulphur or carbon.

2. Compounds according to claim 1, wherein Y is oxygen.

3. Compounds according to claim 1, wherein Y is oxygen and Z is carbon, and wherein n is an integer from 1 to 3.

4. Compounds according to claim 1 wherein Z is sulphur.

5. Compounds according to claim 1 wherein Y is sulphur and wherein n is an integer from 1 to 3.

6. Compounds according to claim 1 wherein Y is oxygen, Z is sulphur and wherein n is an integer from 1 to 3.

7. The compound 3-(3-trifluoromethylphenyl)-1,3-benzoxazine-2,4-dione.

8. The compound 6,8-dibromo-3-(3-trifluoromethylphenyl)-1,3-benzoxazine-2,4-dione.

9. The compound 6,8-dibromo-3-(1,3-dichloro-4-trifluoromethylphenyl)-1,3-benzothiazine-2,4-dione.

10. Compositions comprising at least one compound according to any of the preceding claims, together with an inert pharmaceutical diluent.

11. Compositions comprising at least one compound according to any of claims 1 to 9 together with a soap and/or detergent, both as hereinbefore defined.

12. Compositions comprising at least one compound according to any of claims 1 to 9 together with plastics and/or rubber.

13. Fibrous materials whenever impregnated with at least one compound according to any of claims 1 to 9.

14. Compositions according to claim 11 wherein the total weight of said compounds is in the range 0.001% to 5% of the total weight of the composition.

15. Compositions according to claim 12 wherein the total weight of said compounds is in the range 0.005% to 0.5% of the total weight of the composition.

16. Fibrous materials according to claim 13 wherein the total weight of said compounds is in the range 0.01% to 0.5% of the total weight of said impregnated materials.

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Leamington Spa: Printed for Her Majesty's Stationery Office, by the Courier Press.—1968. Published by The Patent Office, 25 Southampton Buildings, London, W.C.2, from which copies may be obtained.